Occurrence of Macrofungi In Burned and Unburned Hurricane-Impacted Loblolly Pine Forest[1/]

by

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ABSTRACT

Biomass production is limited by the availability of nutrients. Because the nutrient bank of an ecosystem is fixed, the rate at which nutrients are released from dead plant biomass is a major determinant of forest productivity. The effects of recurrent low-intensity prescribed fire upon the rate of decay of large fuels are not well understood. The passage of Hurricane Hugo over the Francis Marion National Forest in September, 1989 superimposed an abundant supply of large-diameter fuels on a long-term winter burning study. We took advantage of this opportunity to explore the relationship between various dormant-season burning intervals and macrofungi in this mature loblolly pine (Pinus taeda)/pond pine (P. serotina) stand in coastal South Carolina.

Fruiting bodies (FB) were sampled on three 2-acre plots, one that has been burned annually, one burned triennially, and one unburned since the original study was installed in 1958. Collections during March, 1991 showed a biomass range from 94 grams on the annual to 981 grams on the triennial plot. Under the influence of the humid, hot summer, July FB biomass ranged from a low of 989 g on the unburned plot to 11,655 g on the annual plot. Except for one rare (in our collections) species found only on the unburned plot, over 90% of the FB weight of a species occurred on the burned plots. The most common species were Hirschioporus abietinus, H. pargamenus, and Polyporus guttulas. Coriolus Hirsutus was the only fungi common on the burned, not collected on the unburned plots.

High cellulase activity is thought to be a prerequisite for nutrient cycling, and PPO activity has been associated with the ability of certain fungi to degrade ligno-cellulose complexes. Laboratory procedures to estimate these activities, including problems encountered are described. Preliminary results indicate high levels of cellulase activity on the burned plots in comparison to the unburned controls which suggest close interval prescribed fires may increase rather than inhibit decomposition of large-diameter, sound wood on the forest floor.

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INTRODUCTION

Biomass production is limited by the availability of nutrients. Because the nutrient bank of an ecosystem is fixed, the rate at which nutrients are released from dead plant biomass is a major determinant of forest productivity. The effects of recurrent low-intensity prescribed fire upon the rate of decay of large fuels are not well understood. The passage of Hurricane Hugo over the Francis Marion National Forest in September, 1989 superimposed an abundant supply of large-diameter fuels on a long-term winter- burning study. We took advantage of this opportunity to explore the relationship between various dormant-season burning intervals and macrofungi in this mature loblolly pine (Pinus taeda) / pond pine (P. serotina) stand in coastal South Carolina.

MATERIALS AND METHODS

Collection of Fruiting Bodies

Three of the 2-acre treatment plots (Ckc, 1c and 3b) were selected for collections. One plot (3b) was burned triennially , one (1c) has received annual burns since 1964, and one (Ckc) was unburned since the original study was initiated in 1958 except for a low-intensity growing-season wildfire that burned part of the plot during the summer of 1988 before going out. Each plot was systematically traversed while removing samples of each fruiting body type (FBT) from the residual wood. An estimate of the samples' proportion of the FBT total volume on a piece of wood was made for each fruiting body type within the sampling cohort. This estimated value was used in the final determination of FB biomass (Collected biomass [g] X Volume proportion = Computed biomass). These data are given in Table 1.

Isolation and Maintenance of Stock Cultures

Stock cultures were established from collected fruiting bodies before the samples were oven-dried and weighed . Once a suitable isolate was established the fungus was maintained in culture tubes on malt extract agar (MEA) which contained 25g of malt extract and 15g of agar per liter of distilled water.

These stock cultures were the source of inoculum for Petri dish cultures that were used to make the following determinations.

Growth of Liquid Cultures and Mycelial Maceration

The peripheral region of cultures maintained for 6-10 days on MEA medium were removed from Petri dishes and placed into sterile stainless steel blender cups containing 25 ml of liquid malt extract (ME) medium (Fig. 1). Mycelia were then macerated for 10 seconds and 1 ml of the macerate was placed into each of several 250 ml Erlenmeyer flasks that contained 25 ml of ME. Flasks were placed onto a rotary shaker at room temperature and agitated at 120 rpm. After 3-11 days of incubation, culture filtrate (CF) was collected by passing through filter paper within a vacuum funnel. To determine dry weight, mycelial pellets were placed into aluminum weighing dishes and oven-dried to constant weight. Culture filtrates were saved and used for protein, polyphenol oxidase and cellulase activity determinations.

Protein Determination

The protein concentration for each CF flask was determined by using the Bio-Rad protein assay (Bradford, 1976).

Polyphenol Oxidase Activity

Culture filtrate (0.2 ml) was added to 10 ml tubes containing 1.0 ml of 0.4 Mm 2,6-dimethoxy phenol (DMOP) in 0.05M citrate/phosphate buffer at Ph 4.0 or 6.0. The contents of the reaction tubes were read within the spectrophotometer at 460 nm. Each reaction tube was read upon mixing and at 10 min intervals up to 60 minutes. As controls, CF portions were boiled for 30 minutes prior to mixing with DMOP.

Cellulase Activity

Cellulase activity was determined by measuring the rate at which glucose accumulates when carboxymethyl cellulose (CMC) is incubated within CF. CMC (200 mg) was placed into 5 ml of CF and incubated for 2 hr. The amount of glucose was

determined by using the Somogyi (1951) test for sugar.

RESULTS

Collection of Fruiting Bodies

Collection during March, 1991 showed a biomass range from 94 grams on the annual (1c) to 981 grams on the triennial (3b) plot (Table 1.). Since there was less than a month between the winter burn of 1c and the March collection, these data will be excluded from further discussion. We, however, think it is important to point out that S. commune, C. hirsutus and H. abietinus are the only identifiable FB collected within burned (1c and 3b) plots.

Table 1. Fruiting bodies collected during March, 1991.

Plot Biomass	Sample (FBT)	Volume Proportion	Collected Biomass	Computed (Total)	Plot
1 c	C. hirsutus	0.30	13.0	43.0	
	Unidentified #9	0.20	10.1	51.0	
	H. abietinus	1.00	0.1	0.1	**94.1**
Ck c	L. betulina	0.01	0.6	60.0	
	C. versicolor	0.05	1.4	28.0	
	Unidentified #10	0.01	1.8	180.0	
	S. commune	1.00	0.5	0.5	
	Fomes	1.00	0.8	0.8	
	C. versicolor	0.01	1.3	130.0	
	Unidentified #7	0.10	0.8	8.0	
	H. abietinus	0.02	1.3	65.0	
	L. betulina	0.10	7.8	78.0	
	C. versicolor	0.20	17.9	90.0	**640.3**
3 b	S. commune	0.01	0.1	10.0	
	C. hirsutus	0.10	4.6	46.0	
	H. abietinus	0.01	0.2	20.0	
	Unidentified #4	0.03	5.5	183.0	
	C. hirsutus	0.04	24.1	603.0	
	Unidentified #8	1.00	11.6	11.6	
	C. hirsutus	0.10	10.8	108.0	**981.0**

Computed Biomass = Collected Biomass/Volume Proportion

Fruiting bodies collected during the humid, hot summer (July, 1991) are listed in Table 2. Three fungi (Polyporus guttulas (Fig. 2), Hirschioporus pargamenus (Fig. 3) and Hirschioporus abietinus (Fig. 4) were found within all plots and were the major contributors of the total fruit body biomass for each plot. These three species accounted for more than 84% of each plot's total biomass. Coriolus hirsutus (Fig. 5) was of much interest since it was only collected from burned plots (3b and 1c). With the exception of H. abietinus, which had more biomass within the check plot (Ckc) than was observed within Plot 3b, there was more FB biomass within both burned plots than within the check plot. The occurrence of H. abietinus within Ckc is considered atypical since a portion of the plot had been exposed to an accidental fire. Fruiting bodies of H. abietinus were only associated with trees that were burned. The total dry weight for all fungi within each plot is presented in Figure 6. The total biomass for the control plot (Ckc) was 939g as compared to 9,183 and 11,655g for plots 3b and 1c, respectively.

Table 2. Fruiting bodies collected during July, 1991.

	Fungi											
	Gut	Albe	Parg	Abie	Gilv	Com	Cinn	Hirs	Stri	Arcu	Nid	Tdti
Plot Ckc												
A	4	13	28	410	2				2			
B	48	3	64	4								
C		3		10								
D				280								
E				100								
F				18								
Total	52	19	92	822	2				2			
Plot 3b												
A	221	264	49	36				550			300	28
B	36		740	380							4	310
C	1880			120								
D	795											
E	3470											
Total	6402	264	789	536				550			304	338
Plot 1c												
A	165		1320	2400			2	560	53	1	600	
B	18		2	4600				70	1		3	
C	142			140					6		4	
D				920					18			
E				350								
F				240								
G				40								
Total	325		1322	8690			2	630	78	1	607	

Gut = Polyporus guttulas
Albe = Polyporus albellus
Parg = Hirschioporus pargamenus
Abie = Hirschioporus abietinus
Gilv = Polyporus gilvus
Com = Schizophyllum commune
Cinn = Pycnoporus cinnabarinus
Hirs = Coriolus hirsutus
Stri = Gleophyllum striatum
Arcu = Polyporus arcularius
Nid = Not identified
Tdti = Too deteriorated to ID

Growth and Enzyme Analyses

Stock cultures of H. abietinus, C. hirsutus, Dichomitus squalens and C. versicolor were grown in liquid malt extract (ME) medium. These cultures

were harvested within 4-6 days and their extracts examined for both cellulase and polyphenol oxidase (PPO) activities (Table 3).

Table 3. Values for protein, PPO activity and cellulase activity.

Fungus	Protein (mg ml^{-1})	PPO[a] Units	Cellulase[b] Units
D. squalens	0.0052	171	28
H. abietinus	0.0159	1245	20
C. hirsutus (4 d)	0.0070	71[c]	0
C. hirsutus (6 d)	0.0043	3424[c]	
C. hirsutus (11 d)	0.0070	1775[c]	
C. versicolor	0.0043	2906[c]	

The values are the means when n=3 or 4.
[a] 1 Unit = change of .001 OD (595 nm) min^{-1} per mg protein.
[b] 1 Unit =1 mol glucose 2 h^{-1} mg ml^{-1} protein.
c pH = 6.00

PPO activities ranged from 71 units for C. hirsutus after 4 days in culture to 2906 units for C versicolor. Intermediate values of 171 and 1245 units were recorded for D. squalens and H. abietinus respectively. A time-course study of C. hirsutus indicated that the PPO activity reached a peak (3424 units) at 6 days. Cellulase activity was observed in culture filtrates (CF) of both D. squalens and H. abietinus during the early days (4 days) of incubation. There was no indication of cellulase activity within CF of C. hirsutus during the same period of incubation.

DISCUSSION

Biomass data obtained during the July, 1991 collection suggest that there is a relationship between burning and fruiting biomass. For example, the occurrences of 989, 8,647 and 11,655g of fruiting biomass in the Ckc plot, 3b plot and 1c plot, respectively, indicate that recurrent low-intensity prescribed fire influences macrofungal biomass production. The comparatively low fruiting biomass for all plots during the March collection is not surprising since seasonal variations have been previously reported (O'Halloran et al., 1987). Unlike the July collection, there was more fruiting biomass on 3b than on 1c. We believe that this relatively low biomass production on 1c is because of the short time period (1 month) between burning and FB collection.

Coriolus hirsutus was the only FBT that was not recorded during the July collection on the unburned plot. Polyporus guttulas and Hirschioporus pargamenus occurred on all plots, but produced more biomass on burned than on unburned plots. The biomass data for these macrofungi suggest that burning enhances their occurrence. It has been reported that occurrence of both C. hirsutus and H. pargamenus were significantly influenced by silvicultural treatments (Mayfield et al., 1990). There are indications that the occurrence of H. abietinus is influenced by burning. Even though fruiting biomass was relatively high on the check plot, almost all the FB were on trees burned by a wildfire. Future collections will include several unburned plots, as well as other triennially and annually burned plots, some with a longleaf pine (P. palustris) overstory.

The relatively high cellulase activities for both H. abietinus and D. squalens are of interest since their occurrence seems to be influenced by burning. D. squalens was not discussed in the collection data since it was collected in July, 1992. This collection was not useful due to heavy insect damage that made identification impossible. Both macrofungi occurred on large-diameter pine boles in burned plots.

It is possible that cellulase assay of C. hirsutus will produce

different values when conducted after 6 days. Future enzyme analyses will include both culture and assay modifications.

The experimental design is based upon the rationale that cellulase activity is related to decay activity or saprophytic efficiency (Ahmad and Baker, 1987). High cellulolytic activity is considered a requisite for nutrient recycling. Although not clearly understood, PPO activity has been associated with certain fungi's ability to degrade ligno-cellulose complexes (Coll et al., 1993). The efficiency of nutrient recycling by these fungi will consist of enzyme activity, fruiting body biomass and frequency of occurrence within the plot.

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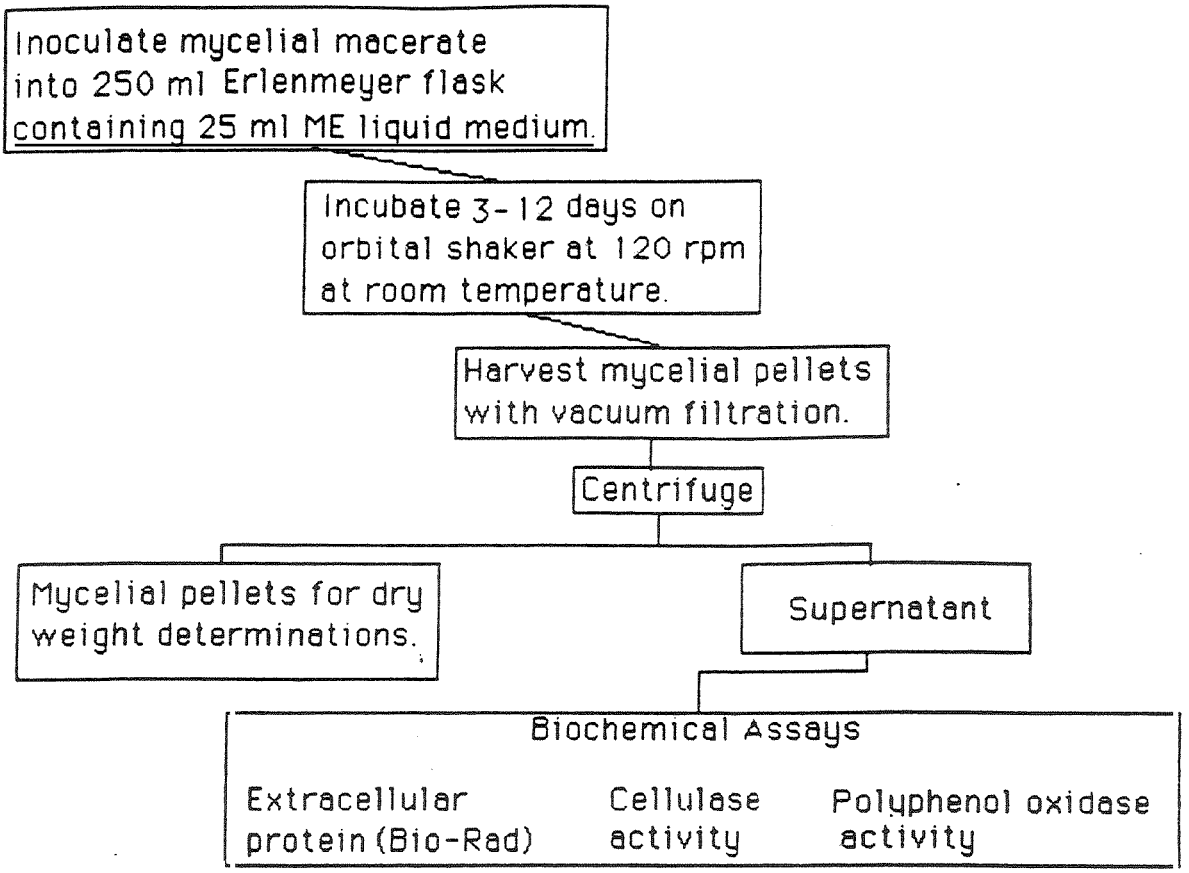

Figure 1. Flow chart of experimental design for determining saprophytic efficiency of certain fungi.

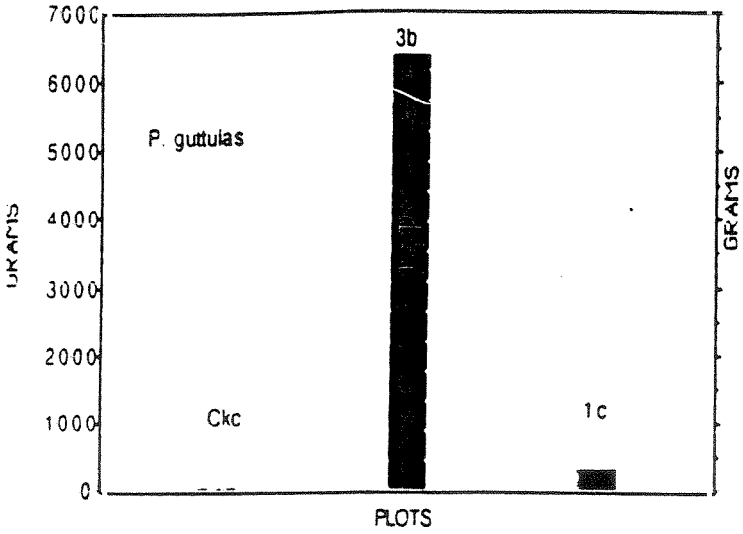

Figure 2. Comparison of *P. guttulas* FB biomass within plots.

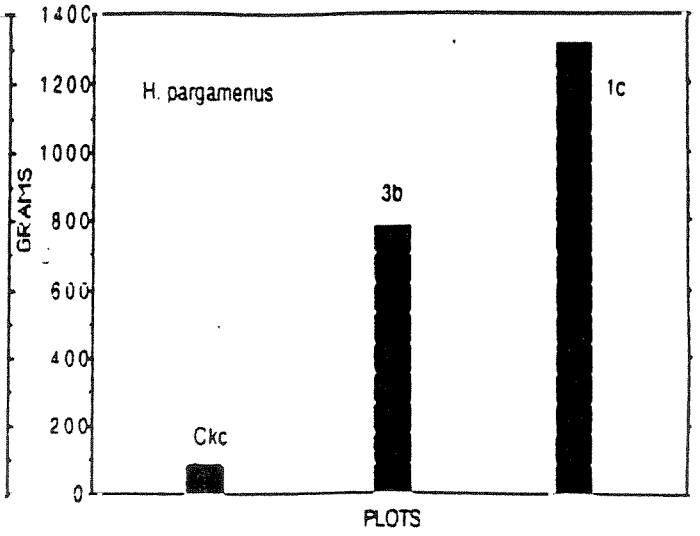

Figure 3. Comparison of *H. pargamenus* FB biomass within plots.

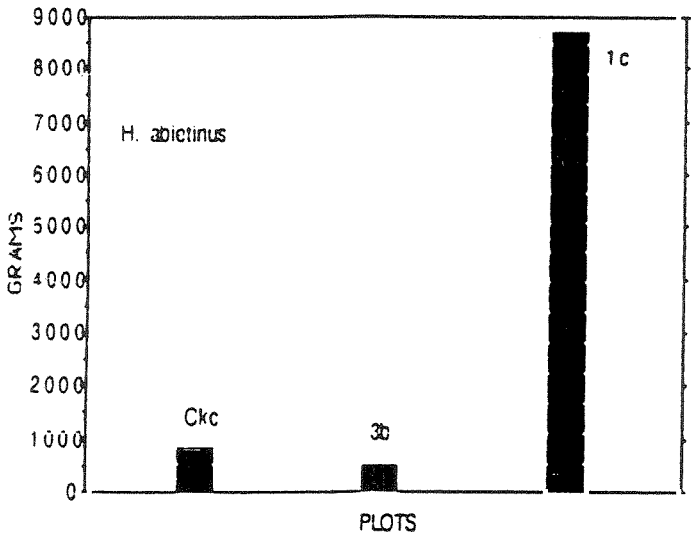

Figure 4. Comparison of *H. abietinus* FB biomass within plots.

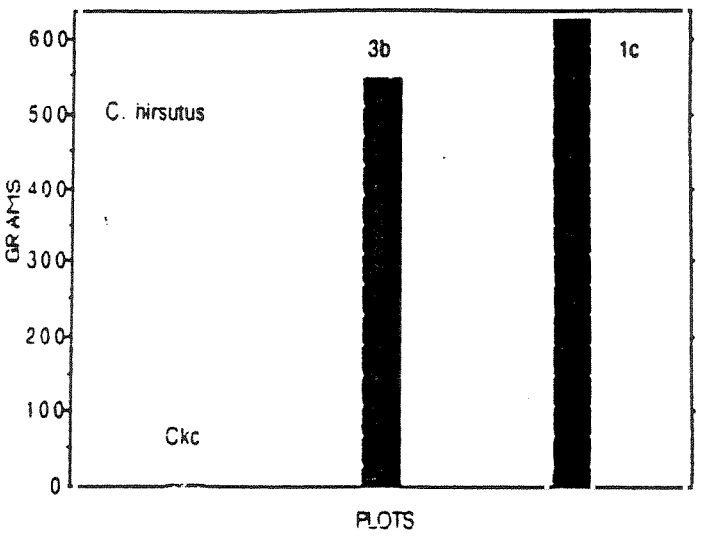

Figure 5. Comparison of *C. hirsutus* FB biomass within plots.

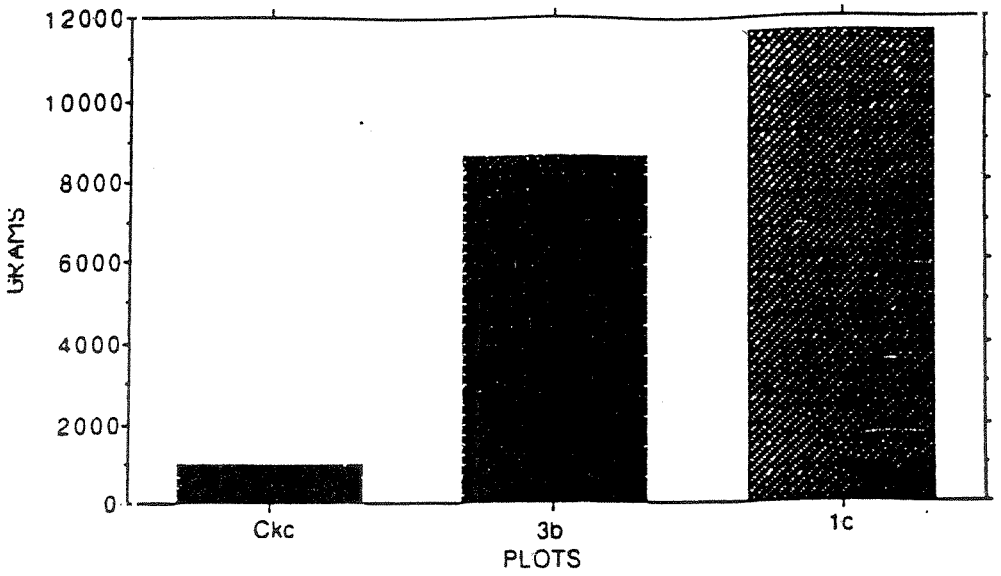

Figure 6. Total FB biomass for all macrofungi within plots.

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